Br. J. Pharmac. (1983), **79**, 053–056

Effect of angiotensin II and 5-hydroxytryptamine on the vessels of the human foetal cotyledon

D.R. Abramovich, K.R. Page* & F. Wright*

Department of Obstetrics and Gynaecology and Department of Physiology,* University of Aberdeen, Aberdeen

1 The actions of angiotensin II (AT II) and 5-hydroxytryptamine (5-HT) on the vessels of the human isolated, perfused, cotyledon were examined *in vitro*.

2 The cotyledonary vessels were shown to respond to both AT II and 5-HT over the range 10^{-8} to 10^{-4} M.

3 The preparation was found to be more responsive to AT II than 5-HT.

4 The findings confirm that the responsiveness of the cotyledonary vessels differs from the vessels of the umbilical cord, and that this behaviour does not depend upon the integrity of the endothelium associated with these vessels.

Introduction

It is known that the foetal vasculature of the human placenta responds to vasoactive drugs (Von Euler, 1939) but the physiological and pharmacological significance of this response is still far from being understood (Boddy, 1979). In early studies the placenta was perfused via the vessels of the umbilical cord. However, the properties of this preparation were found to show considerable variation with the consequence that it was extremely difficult to assess reliably the responsiveness of the vasculature to various drugs (Von Euler, 1939; Eliasson & Åström, 1955).

The vessels of the umbilical cord have been found to give more consistent results and hence have been more thoroughly studied than any other part of the placental vasculature. These studies have been made using the perfused, intact vessels of the cord, and by isometric contraction of muscle strips dissected from the umbilical arteries and vein. Both of these methods have shown that the umbilical vessels are more responsive to 5-hydroxytryptamine (5-HT) than angiotensin II (AT II) (Gokhale, Gulati, Kelkur & Kelkur, 1966; Altura, Malaviya, Reich & Orkin, 1972). Recent evidence indicates this pattern of response is not found throughout the foetal vasculature within the placenta. Using muscle strip preparations Tulenko (1978, 1979) has shown that the villous stem arterioles show a greater response to AT II than 5-HT. Furchgott & Zawadski (1980) however, have demonstrated that the intact endothelium of blood vessels may markedly alter the action of vasoactive agents upon the smooth muscle associated with these vessels. There is therefore a need to confirm Tulenko's findings using a preparation that maintains the integrity of the endothelium. The present work has been undertaken to fulfil this need. Since the pioneering work of Panigel (1968) it has become possible to perfuse the isolated human cotyledon *in vitro*. The present work therefore uses this technique to examine the effects of 5-HT and AT II on the villous stem vessels.

Methods

Normal term placentae were obtained from either vaginal deliveries or from Caesarian sections. In each case the placenta was placed immediately in Krebs solution and transferred with the minimum delay (normally about 30 min) to the perfusion apparatus. The composition of the Krebs solution was (mM): NaCl 118, KCl 4.7, $NaHCO_3$ 25, KH_2PO_4 1.18, $MgSO_4$ 1.06, $CaCl_2$ 2.53 and D-glucose 5.55. Krebs solution was used for both foetal and maternal circulations, the foetal perfusate also containing 50 g l^{-1} dextran (mol.wt. 60,000, Sigma). Perfusates were gassed with 95% O_2 and 5% CO_2 and their compositions monitored using a Radiometer PHM 73 pH/blood gas analyser. Perfusate pH was maintained in the range 7.47 ± 0.04 and Po_2 in the range 556 ± 12 mmHg.

The perfusion procedure was similar to that described by Schneider, Panigel & Dancis (1972) ex-

cept that the placenta was mounted, maternal side downwards, on an open mesh plastic grill inside a water jacketed perfusion chamber. Both placenta and perfusates were maintained at 37±1°C. Flowmeters (Meterate tubes, Glass Precision Engineering Ltd) were sited so as to monitor the arterial supplies to the foetal and maternal (intervillous space) circulations, and the venous return to the foetal circulation. The foetal venous return was also monitored by a drop meter (Devices) connected to a chart recorder (Servoscribe). The hydrostatic pressures of the arterial and venous sides of the foetal circulation were recorded by transducers (Kulite) located at points close to the perfusion chamber, the transducer outputs being connected to a two pen chart recorder (Servoscribe). The foetal flow rate was in all cases within the range 10–14 ml min^{-1} corresponding to an absolute pressure of 98 mmHg on the arterial transducer and 5 mmHg on the venous transducer. The maternal flow rate through the intervillous space was 12 ml min^{-1}.

The drugs employed (all Sigma) were valine angiotensin II, 5-hydroxytryptamine creatinine sulphate and 5-hydroxytryptamine hydrochloride. These were made up in stock solutions of 500 $\mu g\ ml^{-1}$ and diluted with Krebs solution to the required concentration immediately before use. Bolus volumes of 3 ml were administered to the foetal arterial supply by means of a manifold. All measurements were made under conditions of constant arterial flow rate. The drug response was measured by summing the maximum changes in the arterial and venous pressures recorded by the pressure transducers (see Figure 1). Small volumes (3 ml) of foetal Krebs solution were used as controls for AT II; 3 ml volumes of foetal Krebs, and of creatinine sulphate (Sigma) made up in foetal Krebs (10^{-5} M) were used as controls for 5-HT.

Results

Preparations were used only if the foetal venous return was greater than 90% of the foetal arterial supply (normally it was 100%). The equivalent values for the foetal arterial supply and venous return was maintained for periods of about 2 h, after which time there was a progressive decline in the magnitude of the venous return. All drug responses reported here were obtained before the onset of this decline. The base level of the foetal arterial pressure typically increased at a rate of 2 mmHg h^{-1}; if this rate were greater than 5 mmHg h^{-1}, the preparation was rejected. Such preparations invariably showed marked signs of oedema and tended to give abnormal responses.

Tachyphylaxis to both AT II and 5-HT was observed in 60% of the preparations examined. The possibility of tachyphylaxis was minimized by allowing at least 10 min to elapse between the disappearance of a drug response and exposure to another drug or another concentration of the same substance.

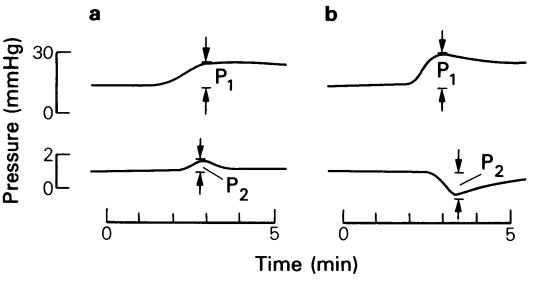

Figure 1 Typical pressure recordings following the administration of a 3 ml 10^{-5} M bolus of angiotensin II (a) during the first 30 min and (b) after 60 min from the start of an experiment. Top and bottom traces represent arterial and venous pressures respectively. The response ΔP was recorded as $P_1 - P_2$ in (a) and $P_1 + P_2$ in (b).

Responses were obtained from both AT II and 5-HT. All responses were vasoconstrictor apart from 4 occasions when 10^{-4} M 5-HT induced a vasodilatation. Figure 1 illustrates a typical response to AT II. Responses produced by 5-HT followed a similar time course. At all times the change in arterial pressure was more than ten times any change in venous pressure. The change in venous pressure was a function of the length of the experiment. During the first 30 min of any experiment, application of either AT II or 5-HT produced an increase in venous pressure (see Figure 1a), this being accompanied by a brief increase in venous return (up to 0.3 ml min^{-1}). Both pressure and flow rate rapidly returned to the basal level following the perturbation. After about 30 min from the start of an experiment, the administration of a drug failed to produce any change in the venous return. When the duration of an experiment exceeded about 1 h, drug administration produced a fall in both venous pressure (see Figure 1b) and venous return (up to 0.5 ml min^{-1}) the effect being fully reversible.

Drug responses over a range of concentrations were obtained by exposing each placenta to either AT II or 5-HT. Apart from a few early experiments with 5-HT, each placenta was treated successively with doses of 10^{-8}, 10^{-6}, and 10^{-4} M. Ten placentae were exposed to 5-HT creatinine sulphate and 3 placentae to 5-HT hydrochloride. No significant differences were noted between the responses produced by these two salts and consequently the data from all 13 placentae exposed to 5-HT has been pooled to produce the curve shown in Figure 2. Figure 2 also shows the curve obtained from 8 placentae treated

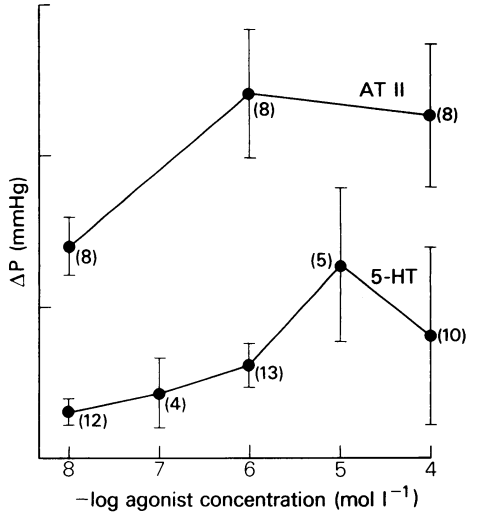

Figure 2 Pressure responses of cotyledonary vessels to 3 ml boluses of either angiotensin II (AT II) or 5-hydroxytryptamine (5-HT). ΔP represents the change in pressure as indicated in Figure 1. Points are means of the number of placentae studied (shown in parentheses adjacent to each point); s.e.mean shown by vertical lines.

with AT II. The error bars shown in Figure 2 indicate that there was considerable variation between preparations, but despite this, the response to AT II was clearly larger than that to 5-HT over the range 10^{-8} to 10^{-6} M. The s.e.mean for 10^{-4} M 5-HT was extremely large owing to the 4 vasodilatations obtained at this concentration. The responses to both drugs tended to increase in the range 10^{-8} to 10^{-5} M.

The responses of a single placenta to both AT II and 5-HT were examined in a second series of experiments. In each experiment the placenta was exposed successively to boluses of drugs at the same concentration, either in the order AT II, 5-HT, AT II or 5-HT, AT II, 5-HT. The first and the third responses of each set were averaged and compared to the second response. In each case the ratio (R) was calculated by dividing the response to AT II by that produced by 5-HT, values of R greater than 1 indicating that the placenta had a greater response to AT II than 5-HT, and values less than 1 indicating the opposite. The results from this series of experiments are summarised in Table 1. It will be seen that 10 placentae were examined using a drug concentration of 10^{-5} M and 10 placentae were studied using a drug concentration of 10^{-6} M. At 10^{-5} M, 7 placentae were found to be more responsive to AT II whilst at 10^{-6} M, 9 placentae showed a greater response to AT II. The value of R was also substantially larger at 10^{-6} M than 10^{-5} M indicating the difference in response to the two drugs was more marked at the lower concentration. The value of R was not dependent on the sequence of drug administration ($F > 0.05$ for 10^{-5} M, $P > 0.10$ for 10^{-6} M).

Discussion

The results summarized in Figure 2 and Table 1 indicate the preparation was more responsive to AT II than 5-HT. The results are in clear agreement with those of Tulenko (1978, 1979). The findings with respect to 5-HT are also in agreement with Panigel (1968), who observed that the cord artery was at least 20 times more sensitive to this compound than the vessels of the cotyledon.

When the present findings are considered with the work of Gokhale *et al.* (1966), Altura *et al.* (1972), and Tulenko (1978, 1979), it is reasonably certain that the responsiveness of foetal blood vessels to AT II and 5-HT varies with anatomical position in the human placenta. It is also of use to note that studies on intact blood vessels give similar results to those on isolated muscle strips. The present studies have been restricted to a pharmacological range of concentrations. However, Tulenko (1978) has observed significant responses to AT II at concentrations as low as 10^{-10} M. This raises the possibility that AT II may have a physiological role in the vessels of the cotyledon although it is too soon to speculate on what this role might be. It is of particular interest in this context that Cooke, Craven & Symonds (1981) have reported the presence of AT II-binding sites in a wide range of placental tissues.

Table 1 Relative responses of placentae to successive doses of angiotensin II (AT II) and 5-hydroxytryptamine (5-HT)

No. placenta studied	*Bolus concentration* (M)	*No. placenta* R > 1	*No. placenta* R < 1	*Mean* R
10	10^{-5}	7	3	1.8
10	10^{-6}	9	1	6.0

(R represents the response ratio $\Delta P_{AT\ II}/\Delta P_{5\text{-}HT}$)

References

ALTURA, B.M., MALAVIYA, D., REICH, C.F. & ORKIN, L.R. (1972). Effects of vasoactive agents on isolated human umbilical arteries and veins. *Am. J. Physiol.*, **222,** 345–355.

BODDY, K. (1979). Fetal blood flow to and from the placenta. In *Placental Transfer*, first edition, ed. Chamberlain, G. & Wilkinson, A. pp. 45–59. London: Pitman Medical.

COOKE, S.F., CRAVEN, D.J. & SYMONDS, E.M. (1981). A study of angiotensin II binding sites in human placenta, chorion, and amnion. *Amer. J. Obstet. Gynecol.*, **140,** 689–692.

ELIASSON, R. & ÅSTRÖM, A. (1955). Pharmacological studies on the perfused human placenta. *Acta pharmac. tox.*, **11,** 254–264.

FURCHGOTT, R.F. & ZAWADZKI, J.V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*, **288,** 373–376.

GOKHALE, S.D., GULATI, O.D., KELKAR, L.V. & KELKAR, V.V. (1966). Effect of some drugs on human umbilical artery *in vitro*. *Br. J. Pharmac. Chemother.*, **27,** 332–346.

PANIGEL, M. (1968). Placental perfusion experiments. *Am. J. Obstet. Gynecol.*, **84,** 1664–1683.

SCHNEIDER, H., PANIGEL, M. & DANCIS, J. (1972). Transfer across the perfused human placenta of antipyrine, sodium, and leucine. *Am. J. Obstet. Gynecol.*, **114,** 822–828.

TULENKO, T.N. (1978). Drug receptor activity in the small blood vessels of the human placenta and their possible significance. In *Fetal and Newborn Cardiovascular Physiology.* ed. Longo, L. pp. 17–32. New York: Garland.

TULENKO, T.N. (1979). Regional sensitivity to vasoactive polypeptides in the human umbilico placental vasculature. *Am. J. Obstet. Gynecol.*, **135,** 629–636.

VON EULER, U.S. (1939). Action of adrenaline, acetylcholine, and other substances on nerve-free vessels (human placenta). *J. Physiol., Lond.*, **93,** 129–143.

(*Received June 30, 1982.*)